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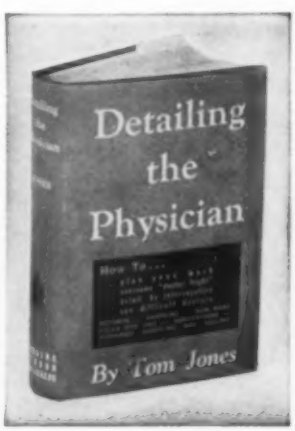

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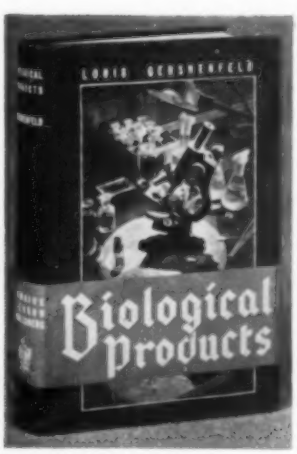

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Studies on Barbiturates. XXV. The Effect of Vitamin C Level on Barbiturate Depression in Guinea Pigs*

By Melvin W. Green† and Rade R. Musulin†

King, *et al.* (1, 2), found that a large series of organic compounds, notably aromatic ketones and alcohols of the terpene series, as well as barbiturates caused an increased excretion of vitamin C in the urine of rats kept on a diet affording minimal vitamin C synthesis. Since the vitamin C content of the tissues did not deviate from the normal, it was concluded that the increased excretion was due to an increased synthesis. Ritz, Samuels and Addis (3) found an increased urinary excretion of vitamin C in rats fed carvone or salicylates. They concluded that feeding of carvone did not deplete the tissues of ascorbic acid while salicylates did. Gruber, *et al.* (4), found an increased vitamin C excretion in rats when fed sodium diphenyl hydantoinate (Dilantin sodium), a compound chemically related to barbiturates; the increased excretion being due to a loss of vitamin C from the tissues, notably the adrenals and the liver.

These findings suggested the following problems: (*a*) Is there a correlation between the vitamin C level of the diet and the degree of depression produced by barbiturates in animals depending entirely on dietary sources for their vitamin C supply? (*b*) In such animals, or humans, on a diet just barely sufficient in vitamin C, would the oft-repeated administration of certain barbiturates produce scorbutic states of various magnitudes? If a correlation between vitamin C level was found to exist, it was hoped that these studies might throw some light on the more general problem of the mode of action of vitamin C as a detoxifying agent.

EXPERIMENTAL

(*a*) *Effect of Vitamin C Level on Barbiturate Depression.*—For these experiments, healthy guinea pigs, weighing between 250 and 300 Gm., were fed a basal diet completely devoid of vitamin C, but complete in all other respects (5). After the animals ceased to gain weight, they were divided into groups as nearly balanced as possible with respect to weight and general health. One of these groups was used as a negative control and received the basal diet only, while the other groups received 0.25, 0.5, 1.0 and 2.5 mg. of pure vitamin C daily by mouth. It will be recalled that 0.5 mg. of

* This investigation was made possible by a grant from Hoffmann La Roche, Inc., which partially defrayed the expense of this study. Presented to the Scientific Section, A. PH. A., Detroit meeting, 1941.

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vitamin C daily is just sufficient to maintain weight and permit no gain; 1.0 mg. of the vitamin will permit virtually normal growth. Each vitamin group was divided in turn into three groups, one of which served as a control and received daily intraperitoneal injections of 1 cc. of saline, while the other groups were given sodium pentobarbital (Nembutal) and sodium phenobarbital, respectively, daily by the intraperitoneal route.

Since the entire experiment involved a total of 50 animals, the determination of the mean sleeping time was not considered expedient and a system of scoring numerically the degree of depression was devised. If animals showed no deviation from the normal, a score of 0 was given, while the presence of general lassitude was awarded a score of 1. If the animal could not maintain its normal posture and assumed a lateral position, a score of 2 was awarded. Complete abolishment of the righting reflex was scored 5. The ability to locomote also was scored as 1, 2 or 3, depending upon the degree of incoördination observed. Using such a scoring method, the highest possible degree of depression was represented by a total score of 11. At no time did any of the animals lose its corneal reflex from the doses of barbiturates employed. The injections and observations were made daily for a period of 30 days.

Table I.—Effect of Vitamin C Intake on Sodium Pentobarbital[a] Depression in Guinea Pigs

Number of Animals	Daily Intake of Vitamin C (Total Mg.)	Average Degree of Depression at 30 Min.	Average Degree of Depression at 60 Min.	Improvement in Animals During Second 30-Min. Period in Per Cent
4	0.0	9.25	9.9	−1.1
4	0.25	9.9	7.3	26.3
4	0.5	6.6	3.9	40.9
4	1.0	7.0	2.7	61.4
4	2.5	5.5	1.8	67.3

[a] 15 mg./Kg.

Table I shows the results of the experiment with sodium pentobarbital given in doses of 15 mg.[1] At the end of 30 minutes after the time of injection, the animals on the higher vitamin levels were not so depressed as the ones on the lower vitamin intake, but the difference was not a marked one. At the end of 60 minutes, however, the difference was more significant. It will be noticed that the negative controls were actually slightly more depressed at the end of 60 minutes than they were at the end of 30 minutes. In the last column of the table, the degree of recovery during the second 30-minute period is expressed in terms of percentages, *i. e.*,

$$\frac{(\text{av. depression at 30 min.} - \text{av. depression at 60 min.}) \times 100}{\text{av. depression at 30 min.}}$$

From these data it is plain that the lower the vitamin C level, the greater the depression and the more protracted the recovery.

Table II shows a similar experiment with soluble phenobarbital administered in doses from 15 to 50 mg. Regardless of the dose of phenobarbital used, it will be noticed that it is almost consistently true that the higher the vitamin C level, the less the depression. Since phenobarbital is slower to take effect and has a longer duration of action than pentobarbital, the time relations are more difficult to correlate with the vitamin level. However, in those cases where extended observations were made, the animals on a high vitamin C level recovered more quickly. Small doses of phenobarbital were used because accumulation was expected, particularly in animals on the lower vitamin levels. However, no observable accumulation occurred at any level up to doses of 50 mg. of phenobarbital.

At the end of the experiment, two of the animals in the phenobarbital series and one in the pentobarbital series which were receiving 0.25 mg. of vitamin C daily, were now given 2.5 mg. of the vitamin daily for 7 days. As the vitamin supply was thus restored, the response to both barbiturates became less severe progressively. For example, the score representing the depression of the animal receiving daily injections of pentobarbital progressively fell from 11 to 5 as the vitamin was deposited in the tissues under the influence of the higher vitamin feeding.

Table II.—Effect of Vitamin C Intake on Sodium Phenobarbital Depression in Guinea Pigs

Number of Animals	Daily Intake Vitamin C[a]	Responses to Varying Doses of Sodium Phenobarbital[b] 15 mg. (7 Days) After 30 Min.	15 mg. (7 Days) After 60 Min.	30 mg. (10 Days) After 30 Min.	30 mg. (10 Days) After 60 Min.	40 mg. (10 Days) After 30 Min.	40 mg. (10 Days) After 60 Min.	50 mg. (4 Days) After 30 Min.	50 mg. (4 Days) After 60 Min.
4	0.0	4.3	4.8	5.3	7.6	[c]	[c]	[c]	[c]
4	0.25	2.6	3.0	3.9	4.3	6.8	6.5	5.1	7.1
4	0.5	2.8	3.6	2.9	2.7	3.7	2.9	6.5	6.7
4	1.0	1.9	2.0	1.2	0.9	1.8	1.9	2.5	2.4
4	2.5	1.1	1.9	0.9	0.6	1.6	2.2	3.0	2.8

[a] Total mg.
[b] Mg. per Kg.
[c] All animals dead.

[1] Throughout this paper, doses of barbiturates are expressed in terms of mg. per Kg.

(*b*) *Effect of Barbiturate on the Vitamin C Level.*—It was thought that the repeated administration of barbiturates might deplete the tissues of vitamin C to such an extent that animals on a bare subsistence vitamin intake would develop a scorbutic state. This did not appear to be the case, for the growth

curves of animals on either barbiturate were substantially the same as those of the saline controls. Furthermore, the vitamin C content of the adrenals and the livers of many of the animals were determined by the indophenol titration method (16). The vitamin content of these tissues was essentially the same in both groups. These results are in agreement with other data in the literature. Therefore, it seems improbable that in humans on a low vitamin C level, the frequent administration of barbiturates would cause an increased vitamin requirement.

There is considerable evidence in the literature that vitamin C may play a role in the detoxification of many types of organic compounds (3, 4, 6, 7, 8, 9, 10). One way in which vitamin C could be of assistance in the detoxification of barbiturates is by the formation of a simple barbiturate-ascorbate complex. To test such a possibility, the vitamin C content of the guinea-pig urine was determined before and after mild acid hydrolysis and was found not to vary. Since mild hydrolysis made no more vitamin available, it was concluded that no such complex was eliminated, at least in a manner analogous to glycuronate conjugation, unless the complex was capable of reacting with the indophenol dye used for titrating the urine. King, *et al.*, observed similar results with albino rats (1, 2).

Because vitamin C is a sugar acid lactone, it is conceivable that the tissues can convert it in part to a glycuronic acid. Since glycuronic acid is widely recognized as a detoxifying agent, it was thought possible that the vitamin detoxified barbiturates through glycuronate complex formation. To test such a hypothesis, the reducing substances of guinea-pig urine of barbiturized animals were determined by the Scales (11) method. The reducing substances present, however, were substantially accounted for in terms of ascorbate itself.

On the basis of the last two findings, any conjugative mechanism for the detoxification of barbiturates must be denied or it must be assumed that the conjugation takes place in the tissues and that the conjugates are so labile as to preclude their presence in the urine.

DISCUSSION

The data presented in this paper show that there is a definite correlation between the vitamin C intake and the response of guinea pigs, animals unable to synthesize the vitamin, to pentobarbital and to phenobarbital. At present, the explanation for this correlation, however, is a difficult if not an impossible task, for the specific mechanism of action of vitamin C and the modification of metabolic processes by this vitamin are unknown. Although the vitamin is capable of undergoing reversible oxidation and reduction, it has never been definitely identified with any specific enzyme system in animal tissues. The work on rats referred to above, showing that the feeding of barbiturates causes an apparent increase in the vitamin C synthesis by the tissues, makes it permissible to assume that this vitamin plays some role in the detoxification of barbiturates. Our finding on guinea pigs would lend support to this assumption if an exhaustion of the animal's vitamin depot would accompany oft-repeated administration of barbiturates. However this was not the case. Since the vitamin was not depleted from the tissues, it can be inferred that the frequent administration of barbiturates to humans is not likely to produce scorbutic states. This is further suggested by the findings of Wright, *et al.* (12), that the daily administration of 180 mg. (total dose) of phenobarbital to humans produced no change in the vitamin C concentration of whole blood, plasma or urine. The urine of our barbiturate-treated animals contained no abnormal amount of reducing substances such as sugars or glycuronates.

Koppanyi, *et al.* (15), have stated that animals in a good physiological condition can destroy more barbiturates than when they are in a state of "ill health." In observing individual animals used during this experiment it was apparent that the animals appearing to be less vigorous and in ill health were more severely depressed by the barbiturates and that the depression lasted appreciably longer. Indicative of such an empirical interpretation, Hjort, *et al.* (13), found a correlation between the mean sleeping time of mice, under the influence of a synthetic ureide, and the completeness of the diet. The nature of the deficiencies of their diets was not stated, but some were assumed to be deficient in protein, vitamin B complex and perhaps calcium.

Until the specific action of vitamin C is better understood, one is not justified in assigning a definite role to vitamin C in the detoxification of barbiturates; one must be content with stating that the general physiological state of animals is an important factor in modifying the action of hypnotics.

When this paper was completed, our attention was called to a note by Keuter, Richards and Klatt (14) on the action of barbital, pentobarbital and pentothal in vitamin C-depleted guinea pigs. These authors stated that at different levels of C avitaminosis the sleeping time from pentobarbital was markedly prolonged while that from barbital or pentothal was little affected. These authors suggest that general metabolic disturbances and changes in the liver following the C avitaminosis are responsible for the prolongation of pentobarbital narcosis. They suggest that the liver is particularly instrumental in the destruction of the short-acting barbiturates, but not of the long-acting group, pentothal being a notable exception. Thus two groups of investigators using slightly different methods of approach have found that lack of vitamin C leads to a prolongation of sleeping time in pentobarbital narcosis. Here unfortunately the agreement stops because the above investigators report that a long-acting barbiturate, barbital, is not appreciably influenced by lack of vitamin C, whereas we found that another long-acting barbiturate, phenobarbital, behaves like a short-acting barbiturate under identical conditions.

Moreover, there is some reason to question the validity of the conclusion of the above investigators because the main difference that we found between the action of barbiturates at a high and at a low vitamin C level is not so much concerned with the prolongation of sleeping time as with the magnitude of the initial depression. We are inclined to believe that a given hypnotic has a greater initial effect in animals at a lower physiological level than in animals at a higher physiological level. These initial differences were very readily observed in the individual animals.

SUMMARY

1. There is a correlation between the vitamin C level and the response produced by phenobarbital and by pentobarbital in guinea pigs. The higher the vitamin level, the less the depression produced by the barbiturates.

2. The frequent administration of pentobarbital and phenobarbital to guinea pigs did not cause a depletion of vitamin C from the tissues.

3. It is suggested that the most likely cause for the effect of vitamin C level on barbiturate depression is the altered general metabolism produced by the lack of this vitamin rather than an effect produced in major degree by direct conjugation.

The authors wish to thank Dr. C. G. King of the University of Pittsburgh for suggesting this problem, Dr. T. Koppanyi of the Georgetown University School of Medicine for guidance in the selection of the barbiturates and advice on the measurements of barbiturate depression, and Dr. R. D. Shaner, Medical Director of the Hoffmann La Roche, Inc., for his constant interest in the problem and the generous supply of vitamin C.

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Marihuana Investigations. IV. A Study of Marihuana Toxicity on Goldfish Applied to Hemp Breeding*

By Brittain B. Robinson†

INTRODUCTION

It has been shown in previous articles (1, 2, 3) of this series that individual plants or varieties of hemp, *Cannabis sativa*, react differently to the chemical Beam tests and that the resins vary in amount and reaction to the Beam tests with hemp of different varieties, different ages, or hemp grown under different climatic conditions. The results presented suggested a possibility of obtaining by plant breeding methods a variety of hemp that might contain a low amount of the resin. It was not proved in the previous articles that the resins in the different lots of hemp were different in their physiological activity as measured upon animals.

Although significant progress has been recently made in elucidating the chemistry of marihuana resins, the material is of such complex nature that it may require consider-

* In collaboration with the Treasury Department, Bureau of Narcotics, Washington, D. C.

† Agronomist of the U. S. Department of Agriculture, Division of Cotton and Other Fiber Crops and Diseases.

able additional work before any simple chemical assay is available that would be useful in plant breeding work.

The purpose of this article is to discuss studies which have been made to determine if the degree of toxicity of hemp extracts to goldfish was of value for plant breeding work in attempting to obtain varieties with little or no active marihuana content. Although the dog is recognized as the standard test animal for marihuana biological assay work, Duquenois (4) reported using fish in aqueous solutions. If goldfish can be used the ease and expense in making numerous tests needed in plant breeding work would be greatly simplified. Results are presented to indicate that dog and goldfish react somewhat similarly to the same extracts. Further, resinous extracts of varieties grown at one location or in different regions of the United States produce different degrees of toxic activity upon fish.

A calibration of toxicity of varied doses of the same preparation of marihuana was not made but it would be desirable in future investigations to make such a calibration.

EXPERIMENTAL

Method of Testing.—The following method was used to obtain the resinous extracts from the hemp leaves. Eight grams of air-dried powdered leaves were placed in an adapter tube. The lower end of the adapter tube was plugged with a half-inch high plug of cotton to act as a filter. The lower end below the cotton was then closed with a stopper and 40 cc. of acetone were used to immerse the leaves. The upper end of the tube was then closed with a stopper. The tube and similar prepared ones of other samples were then allowed to stand over night or for 17 hours at room temperature which probably varied between 70° and 90° F. during several months of experimentation.

The stoppers were removed from the tubes at the end of 17 hours and the acetone extract filtered through the cotton plug. This acetone was immediately filtered with charcoal (Darco Corporation, New York City, brand) and placed in closely stoppered bottles until used.

It was determined that 2 cc. of the acetone extract was the proper amount to use with 1000 cc. of water for a fish test. Two cc. of pure acetone in 1000 cc. of water did not affect fish when tested as controls. All experiments were conducted in an incubator with full width glass doors which allowed observations. The temperature of this incubator was kept at 28° C. The actone extracts of occasional samples of leaves that did not prove toxic to the fish for various reasons were concentrated by allowing slow evaporation of the acetone from measured test-tubes at room temperatures and by this means much greater concentrations of the extract were obtained. This permitted a concentration of the resinous extracts without using more than 2 cc. of acetone per 1000 cc. of water, thus eliminating the effect of acetone which would have appeared in greater acetone concentrations. Goldfish acclimated to 28° C. temperature varying in weight from 3 to 5 Gm. were used in these experiments.

Studies upon leaf material that was first extracted with acetone by the above procedure and then by petroleum ether distillation showed that the acetone failed to remove about 33% of the extractable resins. However, the remaining third of the resins which were only removed by petroleum ether distillation were much less toxic upon fish than the first two-thirds of the total resins extracted.

Purified Resin Fractions.—In preliminary tests the Bureau of Narcotics, Treasury Department, furnished sample fractions of highly purified resins obtained by molecular distillation and of known physiological activity. These were diluted in the ratio of 1 to 10 with acetone. A fraction which was known to be physiologically active on dogs was found to be toxic to fish, and a fraction, not physiologically active on dogs, was not toxic to fish. The activity shown by the fish when affected by these fractions was similar to that produced on fish when affected by extracts of leaf material.

It may be interesting to record that the extracts made up of the purified fractions were theoretically much stronger than extracts prepared from leaves with cool acetone. However, the toxic affect of the strong fractions was not much more rapid than acetone extract of certain leaves. This may have been due to the fact that it was difficult to emulsify the purified fractions in the water.

Toxicity Differences Due to Variety, Maturity and Region of Growth.—Robinson and Matchett (3) reported results on four varieties of hemp grown in 1939 in Mississippi, Nebraska, Virginia and Wisconsin. Three separate harvests representing progressive stages in the growth of the plants were made on each variety in each state. Acetone extracts of available leaf material from these harvests were tested to determine their toxicity to goldfish. The actual results are shown in Table I. In this table the results presented for the earliest harvest are incomplete as the material from the first harvest was not sufficiently toxic to affect the fish in most instances in the first 24 hours at the concentration used throughout the experiment. As a result only one test was made as it was thought that in relation to the other harvest periods the results were conclusive in showing the general trend of the toxicity in reference to the age of the plants. In other words, the toxicity increases with the age of the plant and this is positively correlated with the petroleum ether extraction made from this same material the results of which were presented in a previous article (3).

Table I.—Showing the Number of Minutes Elapsing before Death Occurred When Goldfish Were Placed in Water Solutions Containing Acetone Extracts of Leaves of Four Different Varieties of Hemp Grown in Four States and Harvested at Three Periods of Growth. The Experiment Was in Triplicate for the Second and Third Harvests

Variety / Test number	Virginia 1st	Virginia 2nd	Virginia 3rd	Virginia Total	Nebraska 1st	Nebraska 2nd	Nebraska 3rd	Nebraska Total	Mississippi 1st	Mississippi 2nd	Mississippi 3rd	Mississippi Total	Wisconsin 1st	Wisconsin 2nd	Wisconsin 3rd	Wisconsin Total	Variety Totals
Third harvest:																	
Manchuria "A"	97	152	196	445	156	230	202	588	1440[a]	420[b]	420[b]	2280	134	68	122	324	3637
Manchuria "B"	161	140	203	504	105	161	190	456	1440[a]	141	288	1869	233	167	233	633	3462
African "C"	135	143	227	505	88	93	158	339	93	77	153	323	90	107	112	309	1476
Kentucky "D"	177	83	112	372	113	96	96	305	90	135	99	324	180	127	155	462	1463
State totals				1826				1688				4796				1728	
Second harvest:																	
Manchuria "A"	249	198	177	624	376	336	321	1033	1440[a]	1440[a]	1440[a]	4320	346	208	166	720	6697
Manchuria "B"	307	249	347	903	420[b]	268	420[b]	1108	1440[a]	1440[a]	1440[a]	4320	420[b]	331	233	984	7315
African "C"	155	305	203	663	308	257	199	764	266	195	346	807	180	113	174	467	2701
Kentucky "D"	103	94	86	283	135	127	101	363	115	141	296	552	149	164	170	483	1681
State totals				2473				3268				9999				2654	
First harvest:																	
Manchuria "A"	No material				420[b]				1440[a]				420[b]				
Manchuria "B"	available for tests				420[b]				1440[a]				420[b]				
African "C"					420[b]				1440[a]				176				
Kentucky "D"					118				1440[a]				420[b]				

[a] Alive at end of experiment—24 hours.
[b] Alive at 420 minutes but dead at 24 hours.

Table I shows the marked differences of toxicity obtained with different varieties. Manchurian "A" and "B" were decidely not as toxic as African "C" and Kentucky "D." As these differences were rather consistent at different locations and at different times of harvest, it indicates that hereditary differences exist in varieties. This is important from a practical control method as it indicates the possibility of obtaining by intensified breeding methods a variety of low marihuana content.

Table I further shows that environment plays an important role in the production of the toxicity. This had been expected and it confirms to some extent the belief that the production of the drug is more pronounced in dry elevated regions than in ones of abundant rainfall. This is shown in the table as Mississippi, the most moist location where the hemp was grown, produced the least toxic plants and Nebraska was selected for the driest and most elevated region and it produced the most toxic plants.

Variety Testing.—In 1940, 60 lots of hemp representing original seed from Roumania, Russia, China, Turkey, Tunisia, Hungary, Italy, Germany, Malta, Manchuria, Kentucky, Wisconsin, wild Connecticut, and selections of imbred material from many of these sources were planted in small plots side by side at Arlington, Virginia. Twice during the growing period leaves were collected from plants growing on these plots. Acetone extracts were made upon the leaves and these extracts were tested upon fish. From these tests two plots representing varieties very toxic to fish and two representing varieties only mildly toxic to fish were selected for further study. Additional material of these four varieties was collected and presented to the Bureau of Narcotics, Treasury Department, for alcoholic and petroleum ether extractions. The ether extracts were submitted to Dr. S. Loewe, Cornell University Medical School, who tested their activity upon dogs. The results upon the four selected varieties are presented in Table II.

This table presents results that indicate that the fluidextracts of the Tunisia variety of hemp are from four to eight times as potent on dogs as the fluidextracts of the other varieties. It is not believed that the differences in the stage of maturity of the varieties accounted for the different degrees of toxicity or potency. The Tunisia and Kentucky varieties were the latest in maturing. Further, it is interesting to note in the table that the two varieties, Tunisia and Wisconsin, both are very toxic to fish,

Table II.—Comparison of the Resins Extracted from Four Varieties of Hemp in Reference to Their Potency on Dogs and Their Toxicity upon Fish

Original Source of Seed	Generations Imbred, Brother × Sister	Toxicity upon Fish: Concentration	Toxicity upon Fish: Life Span	Extractions: Alcohol, %	Extractions: Ether, %	Dog (5) Potency	Solid Basis
Tunisia, Africa	2	1 conc.	Dead in 68 Min.	9.4	7.67	0.12	0.70
Wisconsin (Ferramington)	0	1 conc.	Dead in 267 Min.	8.1	6.39	0.03	0.20
Kentucky	1	3 conc.	Alive 24 Hrs.	6.5	3.51	0.02	0.17
Manchurian	2	3 conc.	Alive 24 Hrs.	5.0	3.61	0.014	0.15

both yielded high in alcohol and ether extractions in comparison with the other two varieties of low toxicity to fish, and low active potency to dogs.

The varieties of hemp listed in Table II would hardly be considered pure genetically. It would appear likely that varieties of hemp of even lower potency upon dogs and with less toxicity to fish might be obtained by further selection and imbreeding the Kentucky and Manchurian varieties.

CONCLUSIONS

Acetone extracts of leaves of different varieties of hemp produce different degrees of toxicity in goldfish. The region of growth and the age of the hemp plants affect the degree of the extract toxicity upon goldfish. Fractions of purified resins of different potency acted upon dogs in a somewhat similar manner as they did on fish. Of four varieties used for both fish and dog tests, the degree of toxicity upon fish and potency to dogs was in the same relationship. It is believed that goldfish testing of hemp resins may facilitate hemp breeding.

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Aliphatic Amines I—A Review*

By Melvin F. W. Dunker† and Walter H. Hartung

An extensive survey of the literature on the simple aliphatic amines has been made in order to determine whether it is possible to make a correlation between structure and biological properties. A vast amount of material has been published, by far the most of it pertaining to chemical and physical properties and to methods of syntheses. The references relating to the physiological effects of the simple aliphatic amines, while numerous, represent only a small part. Since the publication of Trendelenburg's comprehensive review in 1923 (1), two partial reviews have appeared, namely, Hartung's tabulation of toxicity and pressor effects (2) and Tainter's discussion of pressor and autonomic nervous system effects (3). Many of the original reports in the literature are difficult or impossible to correlate because of differences in species, in the state of the experimental animal, dosage, technique, mode of administration and the like.

In view of the increasing commercial importance and availability of many simple aliphatic amines, it seems desirable to be better informed on their physiological properties. Furthermore it would seem presumptuous to attempt to correlate the effect of chemical structure of complex compounds with physiological action, a field in which much of the current research is being done, when the simple bases have been inadequately studied or neglected.

The one property on which good comparative data are available is the effect of amines on the blood pressure on intravenous injection. A few generalizations may be repeated here. The lower members, up to the butylamines according to Barger and Dale (4), or to isopropylamine according to Tainter, are inactive or give no constant response on blood pressure in experimental animals. As the chain is increased beyond this point, the pressor activity steadily increases to a maximum in *n*-hexylamine and then steadily decreases as the chain is lengthened to 13 carbon atoms. From propyl to hexyl, it has been shown that the normal radical is more active than the corresponding iso-chain, although Tainter (3) reports the isopropylamine to have pressor activity while the normal propylamine is inactive. Except for the sec-butylamine which was found to be indifferent (3) and the tertiary amylamine for which pressor activity is claimed (5) no other saturated branched chain amines have apparently been investigated. The primary amines seem to be somewhat more active than the secondary and tertiary amines (1).

The amines as a class appear to stimulate the tone and movement of the isolated intestinal and uterine tissues, the effect increasing somewhat with increasing length of the chain up to C_4 (6). Isoamylamine has an effect which parallels that of adrenalin on smooth muscle (7) although it is much less active, that is to say, it produces an inhibition of the intestine, contraction of the rabbit uterus in all states, an inhibition of the non-pregnant cat uterus and

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stimulation of the pregnant cat uterus. On the other hand, isohexylamine is credited with a stimulation of the isolated intestine (6). As before, the iso-form seems to be less active than the corresponding compound and the primary more active than the secondary or tertiary amines.

All of the amines studied are reported to have an effect on the central nervous system which is quite analogous to that of ammonia. There may be preliminary excitation, followed in large doses by narcosis and paralysis (1).

The amines when administered are not excreted as such, but are metabolized in the body to other products, frequently with the destruction of the aliphatic radical, the amino group forming ammonia or urea (1, 8, 9, 10).

In general there seems to be little or no effect on the various secretions although isoamylamine weakly stimulates lachrymal and salivary secretion in cats (7) and salivary secretion in rabbits (1), while trimethylamine is said to stimulate both salivary and pancreatic secretions (11).

Many amines in small doses produce no effect on the body temperature, but according to Trendelenburg (1) trimethylamine is supposed to produce a fall in body temperature in rabbits while the isomeric *n*-propylamine causes a rise in body temperature in rabbits (5).

The replacement of the hydrogens of ammonia with alkyl radicals results in a decrease in the acute toxicity. Beyond this it is somewhat difficult to make generalizations because of the variations in test animals, modes of administration, etc. It appears that there is some decrease in toxicity among the primary amines as the length of the chain increases. In the cases of the methylamines, there is a slight decrease in toxicity in passing from the primary to the secondary to the tertiary amine (1); whereas the reverse is reported for the *n*-butylamines, the mono-*n*-butylamine being least toxic (12). Among the di-alkylamines there appears to be an increase in acute toxicity with increase in molecular weight up to C_8 (1). These toxicity data are not particularly significant since they do not take into consideration the relative molecular weights of the respective amines.

Only a few studies which indicate a chronic toxicity as the result of continued administration of comparatively small doses are reported. For example, when the methylamines, diethyl-, dipropyl-, or isoamylamine (13) were added to the otherwise non-hemorrhagic diet of 7-day-old chicks, hemorrhages, as well as other marked blood changes, occurred. The addition of materials rich in cystine or cysteine to the diet caused a very definite alleviation of the symptoms. Upon subcutaneous injection of 0.5 cc. of $M/15$ solutions of methyl-, ethyl-, propyl- or isobutylamines (14) on five consecutive days into guinea pigs, extensive gastric ulcers were found when the animals were sacrificed on the sixth day. The amines tested showed nearly equal potency in producing the ulcers. When the amines were administered orally with the stomach in the postabsorptive state, the ulcers were more severe. According to Barbour (15), small subcutaneous doses of ethylenediamine, administered daily to rabbits, produce a hypothermia which fails to appear after the fourth day; over a period of ten days such injections have no significant effects on body weight, kidney, heart or blood.

The daily oral administration of 1 Gm. propylamine hydrochloride (about 102 mg./Kg.) to dogs (16) produced no noticeable symptoms while the daily injection of 1-Gm. doses subcutaneously (neutralized) led to necrosis at the site of injection in about 10 days. Propylamine is completely destroyed in the body.

The development of liver lesions in mice has been shown to arise from the continued oral administration of isoamylamine or choline-HCl (17).

On the whole, it appears that there is an absence of characteristic difference between the primary, secondary and tertiary amines. Any differences which appear between the three classes are in degree rather than type of action. When, however, the fourth alkyl is introduced and the quaternary ammonium salts and bases formed, a rather distinct change in character of action appears. The physiological effect becomes predominantly curare-like. The typical action of the amines on the central nervous system is reduced or lost. This loss of central activity on alkylation is also observed among certain of the alkaloids as, for example, strychnine and morphine (1). Recently the high bacteriostatic and bactericidal activities of the quaternary ammonium salts have been emphasized with the marketing of the zephirans, etc. (18).

While speaking of the antiseptic action of the quaternary bases, it might be well to mention that in 1928 Tilley and Schaffer (19) determined the "phenol coefficients" using *B. typhosus* and *S. aureus* for the normal amines from propyl to heptyl, the dialkylamines from ethyl to *n*-butyl and triethylamine. While the phenol coefficient method may be open to question when applied to amines, it can be said that there was an increase in activity in the primary and in the secondary series with increasing chain length, the dialkylamines being more active than the corresponding monoalkylamines. During the past ten years, patents (20) have been issued for the higher aliphatic amines such as octylamine, octyldiethylamine, oleylamine, etc., as disinfectants.

The effect of the presence of halogens in the aliphatic chain has not been investigated fully. It has been shown that tri-(β-chloroethyl)-amine is a very powerful vesicant (21), quite analogous to mustard gas, although the di- and mono-(β-chloroethyl)-amines are not vesicant. Tri-(γ-chloropropyl)-amine and tri-(δ-chlorobutyl)amine (22) are also vesicants.

The introduction of the hydroxyl leads to a decrease in toxicity and activity as compared to the non-hydroxylated amine. Thus ethalolamine is less toxic than ethylamine (1). The very interesting

and useful properties of triethanolamine make it seem desirable to investigate more extensively its physiological properties. Salts of triethanolamine with various iodinated acids (23, 24) have been administered to animals in experiments in iodine therapy without apparent untoward symptoms attributable to the amine.

The introduction of the second amino group in the chain leads to a reduction in the toxicity of the amine (1). This is of interest since the compounds putrescine and cadaverine were at first called ptomaines and thought to be the materials responsible for the severe poisoning resulting from partly decayed food.

While not many unsaturated amines have been investigated, evidence indicates that the double bond leads to an increase in toxicity (1). About 1932, reports on the antispasmodic action of octin (6-methylamino-2-methyl-2-heptene) appeared in the literature and more than 20 references to studies of its action have appeared since that time. The compound is claimed to have strong antispasmodic properties (25, 26), and has been promoted and sold for that purpose. The reports are conflicting. On intravenous injection (27) it causes a loss in tone, a decreased force of rhythmical contraction and a decreased peristaltic activity of loops of the jejunum, ileum or duodenum. This antispasmodic or relaxing action of octin is unexpected in view of the fact that the lower saturated amines are generally reported as stimulating intestinal tissue, causing increased tone and activity. It produces a temporary fall in blood pressure (28) followed by a prolonged rise, as the result of a cardiac stimulant action. The blood-pressure effect is not observed when the drug is given subcutaneously or orally (29), but a rise (30) is reported on intramuscular injection. It has poor bronchodilator activity (31). According to some more recent work (32), the action is weak and irregular on artificial cardiospasm in rabbits, and on the acetylcholine spasm (33) of rabbit intestine. Comparative studies (34) with a series of related saturated and unsaturated amines, either containing or devoid of an *N*-methyl group and having 6 to 9 carbon atoms, indicate that octin is not the optimum compound of the group.

In connection with antispasmodics, some of the recent work of Blicke (35) deserves to be mentioned. Much of his work has dealt with cyclic or mixed amines, but he has studied some aliphatic amines. Methyl-di-β-cyclohexylethylamine [CH_3N—(CH_2—CH_2—C_6H_{11})$_2$] is a strong antispasmodic. If the rings are opened in the 1-2 position, methyl-dioctylamine is obtained. It, as well as methyl-di-*n*-hexylamine, are only weak antispasmodics while methyl-di-(2-ethylhexyl)- and methyl-di-(2-ethylbutyl)-amine have no antispasmodic properties.

Some studies of the effects of amines on various enzymic processes have been made. In general, the alcoholic fermentation of sugar by yeast (36) is retarded by the free amine but may be unaffected or accelerated by the hydrochloride depending on the amine. Similar studies (37) on the effects on the hydrolysis of starch by ptyalin, pancreatin or malt extract indicate that again the free amines retard fermentation while the hydrochlorides accelerate the process, the effect decreasing with increasing length of the carbon chain. However, no mention is made whether or not these effects are associated with the pH of the solution.

A large series of amines, among them 8 aliphatic amines ranging from C_1 to C_{17}, was investigated (38) for their antigenic properties. When the amines were injected into rabbits, they caused the formation of precipitating and complement-fixing antibodies which reacted more or less specifically with the amine used for injection. The effect was weak with amylamine, increased with hexyl- and heptylamines and was pronounced for heptadecylamine.

The toxicity of a large number of aliphatic amines to paramecia, as a measure of their effectiveness against protozoa, has been determined (39) and some of the generalizations follow. Methylamine is only slightly more toxic than ammonia, the toxicity increasing with the number of carbon atoms. Of the amines with the same number of carbon atoms, the isomer with the longest possible primary alkyl chain is more toxic than either the branched chain primary amine or than the secondary or tertiary amine with the same number of carbon atoms; for example, *n*-hexylamine is many times more toxic than isohexylamine, than di-*n*-propylamine or than triethylamine, etc. Most of the aliphatic amines are, however, inferior to quinine in this respect. On the other hand, in a limited series of amines, the compounds with the smallest chain were the most toxic to amoeba (40). Recently, 1,10-tetrabutyl- and 1,10-tetraamyl-diaminodecane (41) have been patented as amœbacides.

Various other effects are attributed to aliphatic amines in the literature as the following selected references indicate. Some aliphatic amines (42) tend to inhibit the growth of tumors and to prolong the life of tumor-bearing animals. Colamine, or ethanolamine, and diethylamine are indifferent whereas choline, triethanolamine, cadaverine and putrescine delayed the appearance of the tumor and prolonged the life period.

Under certain conditions, ethanolamine is reported to prevent the convulsions caused by a vitamin B_1 deficiency (43), whereas methylamine, diethylamine and isoamylamine are entirely ineffective.

The anti-coagulative action of diethylamine is interesting; it is reported to be one-seventh as active as heparin, and, like heparin, its effect is neutralized by hydrochloric acid (44).

Trimethylamine and ethanolamine, when given in doses of 100 mg. per day for twelve days had no effect on the liver fat content of animals with fatty livers as the result of improper diet (45).

The injection of trimethylamine causes a slight fall in the blood sugar level of rabbits (46) whereas methylamine, diethanolamine, ethylamine and ethanolamine are ineffective.

Various amines as methyl-, ethyl-, propyl-, butyl-, dimethyl-, trimethylamines cause swelling, softening and solution of pure casein (47). The effects are attributed to compound formation in consequence of which proteinates possessed of a higher degree of solvent power for water are produced. It is suggested that such a mechanism may account for swellings in pathological states.

According to a recent report (48) solutions of trimethylamine fed to young tomato plants through a cut in the stem for 36 days led to an increase of about 22% in the number of flowers produced. In fish and frogs, the presence of small amounts of trimethylamine in the water leads to the changes in colors associated with the mating period, to moulting, awakening of the sexual instinct and other secondary effects as long as four months after the normal mating season. However, no cases of actual mating were observed. It is suggested that these effects are indicative of hormonal influence.

In view of the fact that the importance of optical isomerism in certain types of physiological action is so well known, it seems remarkable that no reference was found to any studies in which optically active aliphatic amines were prepared and studied physiologically.

It may be seen that while many varied investigations on the biological properties of simple amines have been made, it is impossible to correlate or tabulate the available data and information because of their motley and heterogeneous character. It is obvious that if systematic knowledge in these fields is to become available, properly conducted experiments, carried out under comparable and uniform conditions, must be performed. It is the aim of subsequent papers in this series of "Aliphatic Amines" to record such information.

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Aliphatic Amines II. The *n*-Heptylamines, Preparation and Toxicity*

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With the exception of the primary heptylamine, no studies on toxicity have been made on the C_7 amines. The work on heptylamine is limited to pressor studies (1), studies on the oxidation of heptylamine by tissues (2) and by amine oxidase (3), studies on the antibacterial efficiency (4) of the compound, the production of specific precipitating and complement-fixing antibodies when injected in rabbits (5), and its toxicity for paramecia (6). As a preliminary experiment, it was thought interesting to prepare the amines in pure form and determine whether the position of the amino group in the chain had any effect on the toxicity of the compounds.

The amines were administered intraperitoneally in neutral solutions to white mice. According to the results obtained in comparative experiments, the 1- and 4-aminoheptanes seemed to be the least toxic, the 2- and 3-aminoheptanes being approximately equal but more toxic than the 1-aminoheptane.

EXPERIMENTAL

The amines were prepared free of secondary or tertiary amines by the reduction of the oximes. The necessary aldehyde and ketones were obtained as follows. Eastman technical heptaldehyde was fractionated twice and the cut boiling 152–156° used. Methyl *n*-amylketone and dipropylketone were generously supplied by Carbide and Carbon Company. The samples were fractionated before use. Methyl *n*-amyl ketone boiling at 150–151° and dipropylketone boiling at 142–144° were used. The heptanone-3 was prepared by the dry distillation of an intimate mixture of calcium propionate and calcium valerate. The product was fractionated and a fraction boiling 147–153° used for the preparation of oximes. The oximes were all prepared according to the method given in the literature (7) and illustrated below.

Preparation of Oximes.—To 87 Gm. (1.25 mol) of hydroxylamine hydrochloride in 125 cc. water in a 1-L. 3-neck flask equipped with a reflux condenser, mechanical stirrer, thermometer and dropping funnel were added 114 Gm. (1 mol) heptaldehyde and the mixture was stirred vigorously. A solution of 66 Gm. (0.63 mol) sodium carbonate in 175 cc. water was added slowly so that the temperature did not rise above 45°. The stirring was continued for an hour after the complete addition of the solution. On standing over night, the oxime solidified in large lumps which were filtered out and dried in a desiccator. The yield was quantitative. The crystals melted at 53–55° and after crystallization from 60% alcohol melted at 53.5–54.5°. The preparation of the three ketoximes was carried out in like manner and the properties are given in Table I.

Reduction of Oximes.—Test experiments carried out on the reduction of heptaldoxime in either glacial acetic acid or alcoholic hydrochloric acid in the presence of palladium on charcoal at atmospheric pressure or pressures up to an initial pressure of 750 lb. were not successful in the production of primary amine.

Since the catalytic reduction did not work successfully under the conditions investigated, the reductions were carried out with sodium and absolute alcohol (8). Nine-tenths mol methyl *n*-amyl ketoxime was dissolved in 1.8 L. absolute alcohol and the solution heated to boiling in a water bath under a reflux condenser. The sodium, cut in thin strips (9.8 mols or 225 Gm.), was added slowly through the condenser. Near the end, the sodium melted to a ball which settled to the bottom and it was necessary to continue the heat to retard the formation of a crystalline mass of sodium ethoxide which trapped globules of sodium. When the sodium appeared to be gone, the cautious addition of water was begun and finally 1.5 L. of water were added. The flask was arranged for distillation and the distillate collected in a mixture of 135 cc. concentrated hydrochloric acid and 135 cc. water. The alcohol and most of the water were then distilled from a water bath under reduced pressure. To the amine hydrochloride crystals were added 500 cc. of 40% solution of sodium hydroxide while cooling. The aqueous layer was withdrawn and the amine dried

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over solid potassium hydroxide. The dry 2-aminoheptane was then fractionated and 60 Gm. boiling 141.8–142.3° at 758 mm. were obtained. The other amines were similarly prepared, and their properties are summarized in Table I.

Table I.—Properties of Aminoheptanes

Oxime	Boiling Point, °C.	Amine	Per Cent Yield	Boiling Point, °C.	M. P., °C. Benzamide	M. P., °C. Picrate	LD_{50}, Mg./Gm. Mouse
Heptaldoxime	53.5–54.5[a]	1-Aminoheptane	65	154.5–155.5 (765 mm.)[b]	34–35	120–121[c]	0.1[d]
Methyl *n*-amyl ketoxime	88–90 (5–6 mm.)[e]	2-Aminoheptane[f]	58	141.8–142.5 (758 mm.)[g]	68–69	97–98	0.06
Ethyl *n*-butyl ketoxime	85–105 (5 mm.)[g]	3-Aminoheptane	71	140–144 (761 mm.)	89.5–90	120–121.5	0.07
Dipropyl ketoxime	85–86 (6–7 mm.)[h]	4-Aminoheptane	65	140–141 (761 mm.)[i]	108.5–109.5	165.5–166.5	0.11

[a] 57–58°, Ponzio, *J. prakt. Chem.* [2], 53 (1896), 432.
[b] b_{760} 154.5–155.5°, Ralston, A. W., *Oil and Soap*, 17 (1940), 89; 153°, Paul, R., *Bull. soc. chim.* [5], 4 (1937), 1121.
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[d] The sample supplied by Sharples Solvents Corp. gave the same value.
[e] b_{13} 99.65°, Simon, I., *Bull. soc. chim. Belg.*, 38 (1929), 47.
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[g] b_{15} 97.5°, Timmermans, J., *Bull. soc. chim. Belg.*, 36 (1927), 502.
[h] b_{772} 196°, Trapesongjanz, *Ber.*, 26 (1893), 1433; b_{760} 195°, Mailhe, *Bull. Soc. Chim.*, [4], 15 (1914), 328.
[i] 139–140°, Noyes, W. A., *J. Am. Chem. Soc.*, 15 (1893), 542; 140–141°, Kishner, *J. Russ. Phys.-Chem. Ges.*, 31, 874.
[j] Chloroaurate melted 77–78.5°; Clarke gave 63–64°, *J. Am. Chem. Soc.*, 21 (1899), 1027.

A sample *n*-heptylamine was generously supplied by Sharples Solvents Corporation. It was fractionated and a sample boiling 154.5–155.0° at 765 mm. taken. The derivatives prepared from it agreed in properties with those of the synthetic sample and when tested on mice, no difference in toxicity could be detected.

The picrates were prepared by adding a saturated solution of picric acid in benzene to a benzene solution of the amine. The product was crystallized from benzene or dilute alcohol. The benzamides were prepared by treating the amine with an equivalent of benzoyl chloride in the presence of a slight excess of sodium hydroxide solution. All except the benzamides of 1-aminoheptane crystallized easily from dilute alcohol. The 1-aminoheptane benzamide persisted in remaining as an oil for some time. However, when an ether solution was chilled in a carbon dioxide ice bath, very fine, small needles formed which after three crystallizations from ether and ether-petroleum ether mixtures melted 34–35°. The various properties are summarized in Table I.

Acute Toxicity Determinations.—Toxicity studies were made for the four aminoheptanes synthesized and for a purified sample of commercial 1-aminoheptane.[1] Aqueous solutions of each amine were prepared of such a concentration that the appropriate dose in mg. per Gm. of mouse was contained in 0.7 to 1.0 cc. These solutions were made neutral to litmus with *N*/1 hydrochloric acid. Injections were made intraperitoneally into white mice of both sexes, weighing 18 to 25 Gm. The animals were observed for three days. Usually death occurred within 15 to 30 minutes after injection. Animals surviving 12 hours did not subsequently die while under observation. From preliminary experiments on three different samples of 1-aminoheptane (Sharples) the LD_{50} was found to be between 0.09 and 0.10 mg. per Gm. body weight. The integrated distribution curve (*b* curve) was extremely steep.

A series of determinations was made on all of the isomers between June 21 and July 12, 1940. Three to five mice were injected with each of a series of five doses (equal logarithmic interval) for each compound. Not all compounds were injected on the same day. The LD_{50} for 1-aminoheptane did not vary more than 10% during the experiments, and it is therefore assumed that no appreciable change in sensitivity occurred during the experiments. From the data obtained, the LD_{50}'s were calculated by Karber's method (9). The synthesized 1-aminoheptane had exactly the same acute toxicity as the Sharples sample. Apparently the 1-amino- and the 4-aminoheptanes had about the same toxicity, while the 2-amino- and 3-aminoheptanes were slightly more toxic. A sufficient number of animals was not employed to determine significant differences.

[1] Obtained from Sharples Solvents Corporation.

SUMMARY

1. The four primary 1 amines from *n*-heptane have been prepared in pure form and characterized.

2. The acute toxicity for white mice by intraperitoneal injection has been determined. The 1- and 4-aminoheptanes are of about equal toxicity whereas the 2- and 3-aminoheptanes are more toxic.

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Determination of Iodine in Desiccated Thyroid*

By Frederick F. Johnson† and Harry A. Nelson‡

In the course of another investigation, the authors have had occasion to determine iodine in various samples of desiccated thyroid and various extracted fractions of thyroid and iodinated protein. For extremely accurate work, and especially when very small amounts of iodine are encountered, a recent modification (1) of Leipert's wet digestion procedure (2) is preferred, but for ordinary purposes we have found that ignition in a simple alkaline fusion mixture free from oxidizing agents will give recoveries of iodine comparable to those obtained by the U. S. P. XI thyroid assay and within three per cent of the calculated theoretical values in the case of known mixtures of low iodine content. Interference by nitrates and nitrites is eliminated and the reagents are simplified.

Using four samples of desiccated thyroid and two standardized mixtures of diiodotyrosine and casein, the proposed procedure was compared with three published procedures: the U. S. P. XI assay, the Burnett and Warkow modification (3) and the Manganese Dioxide Method (4). The latter method employs a manganese dioxide ignition mixture and is particularly applicable to the determination of iodine in the presence of oxidizing metallic catalysts. In a study involving 106 analyses by four individuals, the proposed procedure when applied to thyroid gave recoveries essentially identical with those obtained by the U. S. P. XI assay. Applied to the standard diiodotyrosine-casein mixtures, recoveries were slightly higher than by the U. S. P. method and varied between 97.2 per cent and 98.9 per cent of theoretical.

In a previous paper (4), the degree of acidity at which iodine should be liberated by iodic acid was reported. At pH 2.6 or above, the recovery was depressed slightly and the proper reagent blank was not obtained. At pH values below 2.0, the rate of return of color after titrating was increased. Burnett and Warkow (3), in their modification of the U. S. P. XI assay, state that the uncertain end-point and the blank are eliminated and accurate results obtained when the pH is adjusted to 2.5–2.7 and the temperature to about 33° before the addition of potassium iodide. In view of this criticism, the recovery of iodine from standard quantities of potassium iodate was again investigated.

Effect of pH on the Liberation of Iodine.—The iodine liberated by standard quantities of potassium iodate at different pH values was titrated with sodium thiosulfate. Recovery was depressed when the liberation of iodine was conducted at pH values above 2.55.

A series of standard potassium iodide samples (2.00 mg.) were treated exactly according to the U. S. P. XI thyroid assay, the iodine being liberated at different pH values. At values above pH 2.5, the solution was adjusted to 33° C. before the addition of potassium iodide. The results in Table I indicate that the recovery and the reagent blank are depressed above pH 2.5.

Table I.—Analysis of Potassium Iodide

pH	Blank as Cc.	Per Cent Recovery	Determinations
2.52–2.69	0.0	95.1– 96.9	8
1.92–1.98	0.25	100.2–100.8	5
1.52	0.30	99.74	2

Using 0.40-cc. portions of 0.005*N* potassium iodate as representing a normal blank, it is illustrated in Table II that iodine recovery from the blank is low at pH 2.1–2.2 (the range usually encountered in the U. S. P. XI assay) and that no recovery is obtained

* A contribution from the Iodine Educational Bureau's Industrial Fellowship at Mellon Institute. Presented to the Scientific Section, A. PH. A., Detroit meeting, 1941.

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Table II.—Recovery from Reagent Blank

pH	Cc. 0.005 *N* KIO_3	Cc. 0.005*N* $Na_2S_2O_3$
2.18	0.4	0.26
2.22	0.4	0.26
2.36	0.4	0.21
2.54	0.4	0.00
2.62	0.4	0.00
2.20	0.4	0.31
2.20	0.6	0.52
2.20	0.8	0.60
2.20	1.0	0.80
2.16	1.5	1.31
2.60	0.4	0.00
2.60	0.6	0.39
2.60	0.8	0.55
2.60	1.0	0.65
2.60	1.5	1.15

from the blank when the pH is increased above pH 2.5.

Proposed Method.—Accurately weigh 1 Gm. of powdered thyroid and place in a 100-cc. crucible of nickel or Corning Vycor glass. Add 5 cc. of saturated sodium hydroxide solution and 5 Gm. of anhydrous sodium carbonate. Mix thoroughly with a glass rod and leave the rod in the crucible. Add 1 to 2 Gm. of shredded filter paper to facilitate drying and to prevent creeping, and dry the crucible and contents over night at 115° C. Place in a heated muffle furnace and maintain the temperature between 500–525° C. for 20 minutes, then cool. Add 60 cc. of distilled water to the sintered mass, heat to boiling, and decant onto a 18½-cm. filter (Whatman No. 2) into a 500-cc. Erlenmeyer flask. Repeat the treatment, wash the char onto the filter and continue washing with hot water until the filtrate measures approximately 250 cc. Add 3 cc. of saturated bromine water and then slowly add 25 cc. of a mixture of equal volumes of phosphoric acid and distilled water. The pH at this step is approximately 3.2 and the bromine color should be evident. Boil until the vapors do not color starch-potassium iodide test paper. (The time required is usually between 5 and 7 minutes.) Add a few crystals of salicylic acid, wash down the sides of the flask, then cool to room temperature in running water. Add 1 cc. of 85 per cent phosphoric acid, 5 cc. of 10 per cent potassium iodide solution, mix, and titrate immediately with 0.005*N* sodium thiosulfate.

Except for the operation of drying the ignition mixtures over night, a series of eight determinations can be completed within three hours. There are fewer opportunities for serious error than with the U. S. P. XI procedure. Using C.P. reagents, the maximum blank which has been obtained is 0.15 cc. of 0.005*N* sodium thiosulfate. For an indicator solution which is free from interfering pink at the end-point, the authors prefer to use 5 cc. of a 0.5 per cent solution of arrowroot starch made soluble by the method of Wolff and Fernbach (5) and preserved with 0.01 Gm. mercuric iodide per liter. The bromine water should be prepared fresh at least once a week to prevent a return of color at the end-point.

Twenty-six assays on desiccated thyroid, after titration with sodium thiosulfate stabilized at pH 10, have fallen within the pH range of 2.20–2.55.

Preparation of Standard Diiodotyrosine Mixtures.—In order to obtain a thyroid-like material of known iodine content, the authors prepared quantitatively two mixtures of diiodotyrosine and vitamin-free casein. The iodine content of the diiodotyrosine was standardized by the Carius method, the Butler and Burdett method (6), and the Parr bomb method determining the iodine both gravimetrically and volumetrically. The diiodotyrosine was found to contain 54.7 per cent iodine (0.4 per cent variation between the three methods). The quantitative dilutions with casein were milled six hours in a pebble mill. The theoretical iodine contents were corrected for the iodine content of the casein, which was found to be 6 parts per million by the method of Matthews and Curtis (1). Theoretical iodine content: Mixture 1 = 0.1912 per cent; Mixture 2 = 0.2182 per cent.

Assay of Standard Diiodotyrosine Mixtures.—The two mixtures were analyzed by four methods, with two or more analysts performing each analysis. Representative results are tabulated in Table III.

Assay of Thyroid Samples.—Four samples of desiccated thyroid which had been analyzed five years previously by the U. S. P. XI procedure were analyzed by the four methods, each by four analysts. In Table IV, only the average values and maximum variations are tabulated. The figures in parentheses indicate the number of determinations composing each average.

Table III.—Assay of Standard Mixtures

Cc. Blank	U. S. P. XI	B. and W.	Proposed	MnO_2
	Mixture No. 1. I = 0.1912%			
0.30	0.187			0.183
0.30	0.188			0.183
0.25	0.190			0.184
0.25	0.191			
0.0		0.176		
0.0		0.177		
0.0		0.180		
0.15			0.188	
0.15			0.189	
0.15			0.189	
0.15			0.189	
Average	0.189	0.178	0.189	0.183
Per cent recovery	98.8	93.0	98.8	95.9
	Mixture No. 2. I = 0.2182%			
0.30	0.208			0.209
0.30	0.210			0.210
0.30	0.211			0.210
				0.212
0.0		0.199		
0.0		0.199		
0.0		0.202		
0.15			0.212	
0.15			0.212	
0.15			0.213	
Average	0.210	0.200	0.212	0.210
Per cent recovery	96.1	91.7	97.3	96.3

Table IV.—Assay of Desiccated Thyroid

Thyroid	U. S. P. XI	U. S. P. XI, 5 Years Previous	B. and W.	Proposed	MnO_2
No. 1.	0.237	0.236	0.230	0.237	0.234
Max. var.	0.002 (3)	0.006 (6)	0.007 (4)	0.004 (4)	0.001 (3)
No. 4.	0.191	0.201	0.187	0.196	0.193
Max. var.	0.003 (3)	0.006 (6)	0.002 (2)	0.008 (5)	0.003 (4)
No. 5.	0.199	0.203	0.196	0.199	0.200
Max. var	0.006 (4)	0.004 (6)	0.001 (2)	0.000 (5)	0.003 (4)
No. 13.	0.217	0.233	0.209	0.219	0.218
Max. var.	0.011 (4)	0.001 (3)	0.006 (4)	0.003 (5)	0.002 (4)

SUMMARY

A simplified method is presented for the determination of iodine in desiccated thyroid. The procedure involves ignition in an alkaline fusion mixture free from oxidizing agents. Interference by the hypochlorite and nitrate employed in the U. S. P. XI procedure is eliminated. Recovery of 97–99 per cent of the iodine is indicated by analysis of mixtures of standardized diiodotyrosine and casein.

The final titration should be performed at a pH within the range of 2.20–2.50, and the assays of thyroid by the proposed procedure fall within this range.

The stable iodine content of desiccated thyroid, stored in the laboratory for five years with no special precautions having been taken, is worthy of note.

Acknowledgment.—The authors are indebted to C. R. Szalkowski for the analyses made in 1935; also to E. Koozin Johnson, and to E. R. Frederick of the Iodine Educational Bureau for collaboration in the analyses. The authors are grateful for the interest and advice of George D. Beal, Assistant Director of Mellon Institute.

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New Pyrazolone Derivatives

*By Francois X. Demers and E. V. Lynn**

Of the hundreds of pyrazolone derivatives that are described in the literature, relatively few contain substituted phenyl groups and none have acetamino or ethoxy radicals on the phenyl. Since acetophenetidine is used in medicine like antipyrine, the introduction of these two radicals into the latter seemed a promising field of investigation. Accordingly, we started out to make some of the ten possible compounds derived by placing $(EtO)(AcNH)C_6H_3$ for C_6H_5 in antipyrine. While none of these has as yet been produced, several new intermediates have been prepared, as well as a nitrophenylpyrazolone.

EXPERIMENTAL

4-Acetamino-3-nitrophenetole (1).—To a cooled solution of acetophenetidine (0.1 mol) in 50 cc. of glacial acetic acid was added gradually a mixture of 6.3 cc. (0.1 mol) of concentrated nitric acid and an equal volume of acetic anhydride. After standing thirty minutes, the whole was poured into 300 cc. of cold water. The lemon-yellow precipitate, after being filtered and dried, had a melting point of 103–104°. Yield 90% of theory.

4-Amino-3-nitrophenetole.—The product from the first experiment (0.1 mol) was dissolved in boiling alcohol, and a solution of 12 Gm. of sodium hydroxide in 15 cc. of water was added. The mixture was boiled under reflux for one hour and poured into 500 cc. of cold water. The red precipitate, after recrystallization from alcohol, melted at 109–110°. Yield 91.8% of theory.

3,4-Dinitrophenetole.—To 35 Gm. (0.192 mol) of 4-amino-3-nitrophenetole were added 88 cc. of fluoboric acid (2), and to the well-cooled mixture, stirred mechanically, was gradually added a cold solution of 13.2 Gm. of sodium nitrite (0.192 mol). The precipitated diazonium fluoborate, suspended in

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150 cc. of water, was added gradually with stirring to a mixture of 153.6 Gm. of sodium nitrite, 30 Gm. of powdered copper and 300 cc. of water. The resulting precipitate was filtered off, washed with a solution of sodium hydroxide and then with water, and dried at 110°. After recrystallization from alcohol, the buff-colored product melted at 76°. Yield very poor. Per cent of nitrogen found, 13.69; calc., 13.21.

4-Acetamino-3-aminophenetole.—A thin paste of 44.8 Gm. (0.2 mol) of 4-acetamino-3-nitrophenetole and 265 cc. of hot water was poured in a thin stream into a boiling solution of 388 Gm. of ferrous sulfate in 750 cc. of water. Stronger ammonia water was then added with vigorous shaking to definite alkalinity. After boiling the mixture for five minutes, it was filtered and the filtrate was cooled in ice-water to produce 16.6 Gm. of product melting at 139–140°. Concentration of the mother liquor gave more to bring the total to 22.1 Gm., a yield of 54.3%. The reduction was also performed with aluminum amalgam, with ammonium sulfide and with sodium hydrosulfite, but the yield in each case was smaller. Per cent of nitrogen found, 13.97; calc., 14.43. Hydrolysis of the compound by usual methods gave 3,4-diaminophenetole melting at 71–72°.

3,4-Diacetaminophenetole.—A small quantity of 4-acetamino-3-aminophenetole was heated with an excess of acetic anhydride for a few minutes, then an equal volume of water was added, and the mixture was boiled to expel excess of acid. Upon cooling, white crystals were deposited that melted at 186°. Hydrolysis of these, as in the previous experiment, gave 3,4-diaminophenetole melting at 71–72°.

2-Amino-4-nitrophenetole (3).—*o*-Phenetidine (30 cc.) was acetylized with acetic anhydride in the usual way. The product was dissolved in 40 cc. of glacial acetic acid, and 87 cc. of sulfuric acid were added gradually with cooling. To the mixture at 0° was added in small portions a solution of 24 cc. of nitric acid in 36 cc. of sulfuric acid. The whole was then poured into 600 cc. of water, and the resulting precipitate of the acetamino compound, after filtering and washing, was hydrolyzed by heating with hydrochloric acid. This brownish yellow hydrochloride was identified by converting to the amine, melting at 97°.

2-Ethoxy-5-nitrophenylhydrazine.—To 35.7 cc. of 2-amino-4-nitrophenetole hydrochloride (0.164 mol), mixed with 30 cc. of hydrochloric acid and 20 Gm. of ice, were added slowly with stirring 11.8 Gm. of sodium nitrite in 24 cc. of water. The diazonium mixture was added to a cold solution of 114 Gm. of sodium sulfite in 246 cc. of water, previously saturated with sulfur dioxide. The mixture was heated at 60–70° and then for an equal period after adding 26 cc. of hydrochloric acid. After standing over night and further heating, the solution was filtered and an excess of sodium hydroxide was added to precipitate the base, yellow crystals melting at 124.5–125.5°. Yield 30.8% of theory. Per cent of nitrogen found, 19.95; calc., 21.32. Benzaldehyde 2-ethoxy-5-nitrophenylhydrazone, glistening orange crystals melting at 169–169.5°. Acetone 2-ethoxy-5-nitrophenylhydrazone, minute orange crystals melting at 123.5–124°.

1-(2-Ethoxy-5-nitrophenyl)-3-methyl-5-pyrazolone.—A mixture of 6.5 Gm. of 2-ethoxy-5-nitrophenylhydrazine and 6 cc. of acetoacetic ester was heated on a water bath for thirty minutes. The dark red solution solidified on standing to a brown mass, from which 8 Gm. of yellowish crystals were separated, melting at 82–83.5°. Yield 89% of theory. The analysis for nitrogen gave very low results and it is probable that the product is contaminated.

SUMMARY

In attempts to make acetaminoethoxyphenyl derivatives of antipyrine, the following new compounds were prepared: 3,4-dinitrophenetole, m. p. 76°; 4-acetamino-3-aminophenetole, m. p. 139–140°; 3,4-diacetaminophenetole, m. p. 186°; 2-ethoxy-5-nitrophenylhydrazine, m. p. 124.5–125.5°; benzaldehyde hydrazone of the latter, m. p. 169–169.5°; acetone hydrazone m. p. 123.5–124°; 1-(2-ethoxy-5-nitrophenyl)-3-methyl-5-pyrazolone, m. p. 82–83.5°.

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Some of the Constituents of the Tuber of Coqui (*Cyperus rotundus* L.). II. The Volatile Oil

*By Conrado F. Asenjo**

The volatile oil present in the tuber of *Cyperus rotundus* L. is the only constituent of this plant to have been studied chemically in the past. Goebel-Kunze (1) in 1830 reported the presence of a volatile oil in the tuber. The distillation of this oil has been carried out in the Sudan, India and Japan. Its use as a fixative in perfumery has been suggested (2).

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Table I.—Constants of the Volatile Oil of *Cyperus rotundus* L.

Item	Oil from Puerto Rico	Maximum and Minimum Values Reported by Other Investigators	
		Maximum	Minimum
Per cent yield (dry tuber basis)	0.40	1.0	0.45
Density 20° C.	0.9428	1.0944	0.9548
Refractive index 20° C.	1.5070	1.5175	1.4988
Optical rotation	−12.9	+35.5	−19.9
Acid value	1.7	11.3	1.0
Saponification value	22.1	31.4	6.6
Saponification value after acetylation	62.9	105.0	63.3

The first scientific article, devoted to the chemistry of this oil, was written by Joseph and Whitfield (3) in 1922. They determined, the principal constants of an oil obtained in the Sudan. In 1925 Rao, Panicker and Sudborough (4) examined several Indian oils, determining their constants and isolating mixtures of dicyclic and tricyclic sesquiterpenes. Hedge and Rao (5) continued this investigation and, in 1935, isolated for the first time the sesquiterpene-ketone α-cyperone. They also demonstrated the presence in this oil of 1-pinene, cineole and of mixtures of secondary and tertiary alcohols. The structure of α-cyperone has been worked out at Simonsen Laboratory (6, 7). They have also synthesized this compound (8). In Japan Kimura and Ohtani (9) have investigated several samples of the oil distilled in that country.

EXPERIMENTAL

Eight thousand seven hundred and fifty grams of air-dried tubers, obtained in Puerto Rico, yielded 200 Gm. of petroleum ether extractive. This extractive was subjected to steam distillation and 32 cc. of volatile oil were obtained. On cohobating the aromatic water, 3 cc. of oil were recovered, making a total yield of 35 cc., that is, 17.5% of the petroleum ether extractive.

This oil has a faintly camphoraceous terebinthinate odor and a yellow color. The principal constants are reported in Table I.

SUMMARY

1. The literature pertaining to the volatile oil of *Cyperus rotundus* L. is reviewed.

2. The yield and constants of the oil, obtained from Puerto Ricon tubers, have been determined.

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The Chemical Nature of Hesperidin and Its Experimental Medical Use as a Source of Vitamin P— A Review

*By Ralph H. Higby**

The successful clinical treatment of hemorrhagic purpura, and other disorders arising from abnormal capillary fragility, by the administration of hesperidin, has recently been reported by a number of research workers. Present evidence indicates that this glucoside when administered orally fulfils the function of vitamin P, and can probably be regarded as a precursor of this vitamin. Since hesperidin is not generally well known, either to chemists or to the medical profession, it seemed worth while to review the literature dealing with the occurrence, preparation, properties and medicinal uses of this substance.

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OCCURRENCE

Hesperidin is a flavanone glucoside occurring in most varieties of citrus fruit. It was discovered by Lebreton (27) in 1828 and has been made the subject of numerous investigations since that time.

Pfeffer (36) (1874) found that hesperidin was precipitated as sphero-crystals in the cells of the sweet orange by soaking the tissue in glycerine or alcohol, or merely by desiccation. Using isolated pieces of tissue he found hesperidin in pith and bark of young twigs, in leaves and flower parts, in the parenchymatous cells of the fruit flesh and the cells of the pulp. Hesperidin was present in the lime, but not in the grapefruit or the Seville sour orange. He hypothesized that hesperidin occurred in solution in the cell sap from which it was precipitated by dehydration. Tunmann (50) (1913) confirmed this observation. Hall (14) (1925) speculated on the condition necessary for the solution of hesperidin in the cell sap, hypothesizing a glucose-hesperidin complex.

Hesperidin has been reported as occurring in many plants other than citrus. Zenetti (54) (1895) reported hesperidin in *Barosma*, Schulze (43) (1902) in *Skimmia Japonica Thunb*, Vogl (51) (1896) in *Scrophularia nodosa*, Tunmann in *Lobeliaceæ*, *Valerianceæ*, *Umbelliferæ*, *Labatæ*, *Compositæ*, *Papilionaceæ* and *Rutaceæ*. Alverson (1) (1919) found hesperidin in 250 varieties of *Labatæ*, Klein (22) (1921) in *Rubiaceæ*, but only in genus *Galium*, and Nilsson (32) (1921) reported hesperidin in a number of varieties of *Umbelliferæ*.

It is probable, however, that in many of the above instances hesperidin was not adequately identified, and that any substance depositing in the plant cell as sphero-crystals upon desiccation has been reported as hesperidin. Oesterle and Wander (33) (1925) following an extensive investigation found that many of the substances reported in the literature as hesperidin were in reality diosmin.

Hesperidin, according to Tunmann, occurs in greatest concentration in the green fruit. Harvey and Rygg (15) (1936) found a decrease from 3.07% to 2.03% in the rind of Washington Navel oranges in the period from January 5th to April 12th. These analyses are based on the dry weight of peel. Higher percentages were found in the blossom end than in the stem end. It was also shown that hesperidin increased in the fruit during storage.

Iwasaki (19) (1936) determined the amount of hesperidin in Mandarin oranges as follows: dry peel 5.68%, imbedded fiber 5.24%, dry endocarp 1.10%. The juice had only a trace. Kwang-Fong Tseng and Ren Dzin Yu (25) (1936) obtained 8.2% of pure hesperidin by the methyl alcohol extraction of dried orange peel. A related substance, neohesperidin, m. p. 244°, was obtained from bitter oranges by Kolle and Gloppe (23) (1936).

PREPARATION

Most of the early preparations of hesperidin were undoubtedly impure. Lebreton, for example, gave a melting point of 109° for his original preparation. Nearly all of the extraction methods have been similar, depending on the solubility of hesperidin in dilute alkali, and using alcohol to repress the solution of pectin. Typical methods of preparation are given below.

Pfeffer (36) (1874) soaked the crushed fruit in alkaline 50% alcohol for several hours, pressed off the liquor and neutralized with HCl, obtaining sphero-crystals. These were purified by solution in alkali and reprecipitation with acid.

Paterno and Briosi (34) (1876) followed Pfeffer's method of extraction but crystallized from boiling acetic acid, obtaining the first semblance of a pure product, m. p. 243–245°.

Hilger (16) (1876) used a modification of Pfeffer's method on dried green oranges, purifying his product by crystallization from strong alkaline alcohol through saturation with HCl gas, and a final crystallization from acetic acid.

Tiemann and Will (48) (1881) pointed out that many substances reported as hesperidin were mixtures, and that some if not all *Aurantiaceæ* contain another more soluble glucoside. In their preparation of hesperidin the peel was first extracted with large quantities of water to remove lead acetate-pre-

cipitable substances, then exhausted with 50% alcohol containing 1–2% KOH. The hesperidin was precipitated by neutralization, and the crude product cooked out with 90% alcohol to remove colored impurities. The colorless residue was dissolved in very dilute alcoholic NaOH and hesperidin precipitated by bubbling in CO_2. The melting point of the needle-shaped crystals obtained in this way was 251°. Crystallization from alcohol or from boiling glacial acetic acid gave no purer product.

Kwong-Fong Tseng and Ren Dzin Yu (25) (1936) by extracting dried orange peel with a methanol-ethanol mixture to remove impurities, then extracting the residue with large quantities of methanol, obtained hesperidin having the highest melting point yet reported, 261–262° corrected.

CHEMICAL PROPERTIES

Pure hesperidin occurs in long hair-like colorless needles, easily soluble in dilute alkali, and in pyridine, slightly soluble in methyl alcohol and in hot glacial acetic acid, almost insoluble in acetone, benzol and chloroform. In water it dissolves to the extent of one part in 50,000. Hesperidin, in common with other flavanone glucosides, has the property of forming complex crystals with other similar glucosides, which greatly affect its solubility and other physical properties, making it difficult to obtain in a pure state. For this reason much of the earlier work on structure has been misleading. Hoffman (17) (1876) reported that by acid hydrolysis hesperidin was resolved into hesperetin (m. p. 225°) and glucose. Alkaline hydrolysis was said to produce hesperetinic acid. Tiemann and Will (48) (1881) prepared hesperetin by hydrolyzing hesperidin for 3 hours in 2% H_2SO_4 in 50% alcohol, heating in a pressure vessel to 115–120° C. Dilution of the hydrolysis liquor with water precipitates the hesperetin, which is taken up in alcohol, clarified with lead acetate, and recrystallized by dilution with warm water. The crystals melted at 224–226° with decomposition. Analysis showed the formula as $C_{16}H_{14}O_6$. Alkaline hydrolysis of hesperetin yielded phloroglucinol and hesperetinic acid, m. p. 226°. "Hesperetinic acid" was shown to be identical with isoferulic acid. Later Will (52) (1887) determined that both rhamnose and glucose were obtained by the acid hydrolysis of hesperidin, the sugars were separated by the insolubility of phenyl glucosazone in acetone. Tanret (47) (1888) confirmed this finding, and suggested the probable hydrolysis reaction as follows:

(Tanret)

$$\underset{\text{Hesperidin}}{C_{50}H_{60}O_{27}} + 3H_2O \rightarrow \underset{\text{Rhamnose}}{C_6H_{14}O_6} + 2\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Hesperetin}}{C_{28}H_{28}O_{12}}$$

This view was widely accepted and the $C_{50}H_{60}O_{27}$ formula for hesperidin is still given in many handbooks. Perkin (35) (1898) confirmed the formula for hesperetin as $C_{16}H_{14}O_6$, but assigned the substances an ester structure.

Power and Tutin (37) (1907) pointed out the close relationship between hesperetin and homoeriodictyol. Asahina and Inubose (4) (1929) studied the hydrolysis of hesperidin. They found that the reaction proceeded as follows:

(Asahina)

$$\underset{\text{(Hesperidin)}}{C_{28}H_{34}O_{15}} + 2H_2O \xrightarrow{\text{Acid}} \underset{\text{(Hesperetin)}}{C_{16}H_{14}O_6} + \text{glucose} + \text{rhamnose}$$

When heated with $Ba(OH)_2$ hesperidin yielded isoferulic acid plus a rhamnoglucoside. Acid hydrolysis of the latter gave glucose, rhamnose and phloroglucinol.

King and Robertson (21) (1931) confirmed these results and located the position of the sugar residue at carbon atom number seven. The structural formula for hesperidin is that of a typical flavanone as shown below (Fig. 1).

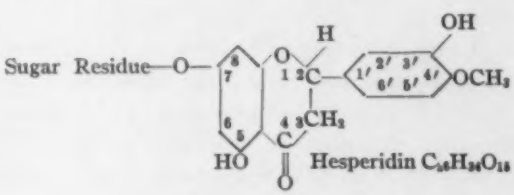

Fig. 1.

Naringin, the bitter glucoside of grapefruit, differs from hesperidin only in the absence of the methoxyl group in position 4′ and the shift of the hydroxyl group to this position (Fig. 2).

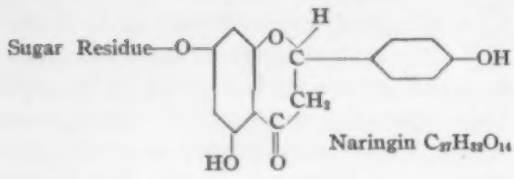

Fig. 2.

The vitamin-like activity of certain lemon peel preparations was believed by Szent-Györyi to be due to eriodictyol glucoside, a substance which had never been isolated, but which could theoretically be formed by the demethylation of hesperidin. The structural formula for this substance is that given by Gortner, shown in Fig. 3.

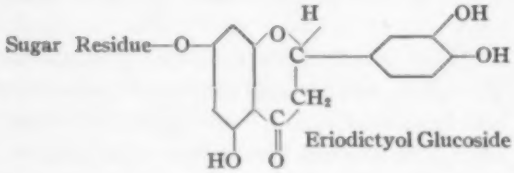

Fig. 3.

MEDICAL USES OF HESPERIDIN

In 1936 Szent-Györyi (3) and a group of his collaborators reported the beneficial effects of lemon juice, and of extracts of Hungarian red pepper, in a number of cases of *purpura hemorrhagica* which had failed to respond to large dosages of pure ascorbic acid. Because of the vitamin-like action of the curative substances in these extracts it was called vitamin P, to indicate its effect on permeability of the capillaries.

Seeking the active principle, a crystalline flavone substance was isolated from lemon juice and was named "citrin." This was shown to be physiologically effective.

Chemical studies of citrin (9, 39) showed it to be a mixed crystalline substance composed largely of hesperidin, but containing smaller amounts of eriodictyol glucoside, called by Szent-Györyi, "eriodictin." This latter was not obtained in pure crystalline form.

Szent-Györyi obtained patents (13, 46) covering the preparation of "citrin" and of "eriodictin" solution, the latter being preferred by him for administration by injection. Chromatographic absorption studies by Robeznieks (38) indicated that citrin contained a third quercitrin-like flavone in addition to hesperidin and eriodictin. This possibly explains the observation of Armentano (2) that citrin brings about a definite lowering of blood pressure which he attributed to a quercitrin-like flavone.

The favorable results reported by various workers in the administration of the several flavone preparations in a large number of clinical cases would seem to establish beyond doubt that these substances exert a valuable therapeutic effect upon capillary fragility and permeability.

In addition to the original observations of Szent-Györyi and his associates, Jersild (20) has reported the curative effect of 50 mg. of citrin daily in treatment of Shönlein-Henoch purpura; Lajos (26) found citrin effective in treatment of kidney hemorrhages; Morii (31) found the administration of 30 mg. of hesperidin daily effective in decreasing capillary permeability and increasing capillary resistance in various clinical cases suffering from pleurisy, tuberculosis, Graves' disease and beriberi; Decker (10) showed remarkable improvement in hemorrhagic diathesis, using dosages of 50–150 mg. daily of citrin, and found that recovery was accelerated by the administration of 500 mg. daily of ascorbic acid. Margitay-Becht (29) has reported a curative effect in three out of four cases of acute glomerulonephritis resulting from dosages of 55–110 mg. of citrin daily. Unfortunately in most of the foregoing work the flavone substances used are not sufficiently described, and are of unknown purity. This is especially true since the term "citrin" has been used loosely to describe either crystalline "citrin" or the "eriodictin" mother liquor.

Perhaps the most significant clinical work on vitamin P has been that of Scarborough and his associates, using hesperidin from oranges as the source of the vitamin. In 1938 Scarborough and Stewart (40) showed the value of hesperidin in the treatment of purpura resulting from the use of arsenicals in the treatment of syphilis. Scarborough (41) (1939) used pure hesperidin, crude hesperidin, soluble "citrin" prepared by Szent-Györyi's method from orange peel, and

orange and lemon juice in the treatment of a number of cases of lowered capillary resistance resulting from generalized vitamin deficiency, with uniformly successful results. Hesperidin was effective when given in doses of 1 Gm. per day orally, the soluble "citrin" was apparently equally effective when 0.2 Gm. daily was injected. Capillary resistance was not increased by administration of ascorbic acid. In a later paper (1940), Horne and Scarborough (18) have shown that erythema and dermatitis resulting from arsenical therapy are caused by lowered capillary resistance. The curative effect of hesperidin was demonstrated in one such case of toxic purpura and in one of toxic erythema.

Kugelmass (24) (1940) has also demonstrated the effectiveness of "vitamin P"[1] from oranges in curing several cases of vascular purpura.

HESPERIDIN AS VITAMIN P

Szent-Györgyi's claim for the vitamin-like nature of the flavone substances, based upon clinical evidence, appeared to be supported by the animal experiments of Bentsath (7) and his co-workers, which showed an increased survival time for those guinea pigs on a scorbutic diet that received supplements of vitamin P concentrates. Zilva (55) was unable to confirm these results, when pure hesperidin, pure eriodictyol, mixtures of these two substances, and citrin prepared according to Szent-Györgyi's directions were administered. Moll (30) likewise reported negative results, and later Bentsath (5, 6) himself was unable to check his earlier experiments. Similarly Detrick (11) failed to show any beneficial effects of citrin in scorbutic guinea pigs.

The negative results obtained by these workers were based upon the failure of the vitamin P preparations to alter the familiar symptoms of experimental scurvy. No attempt was made to measure changes in capillary resistance.

Zacho (53), however, in 1938, using a vacuum cup method, was able to show that diminution of capillary resistance in guinea-pig scurvy was not alleviated by administration of ascorbic acid, but was cured by administration of citrin.

More recently St. Rusnyak and Benko (44), using a similar technique, were able to produce diminished capillary resistance in rats maintained on a scorbutogenic diet, and were able to relieve this condition by the administration of 3 to 4 mg. of citrin per day. Thienes and Leser (49) have also reported a test method using mice as experimental animals.

These results are in accord with those of Scarborough (42) (1940) who has traced the syndrome of vitamin P deficiency in man as contrasted with that of scurvy. He was able to demonstrate a vitamin P deficiency which did not respond to treatment with vitamins A, B, D or ascorbic acid but was relieved by the oral administration of hesperidin and other citrus flavanones containing hesperidin.

These clinical and pharmacological results seem to prove the existence of a vitamin substance of flavone nature which serves to control the condition of the capillaries. Whether hesperidin itself is this vitamin or whether it is merely a precursor which is broken down in the digestive system to form the vitamin is not yet known.

Some investigators have questioned the vitamin nature of hesperidin on the grounds of the high dosage required. This is slightly less than 1 Gm. per day for an adult. It should be remembered, however, that nothing is known of the minimum protective dose. Comparing this curative dose for hesperidin with that of vitamin C (100–1000 mg. per day), it becomes evident that the two are of the same order of magnitude and therefore this argument alone is not valid.

SUMMARY

Hesperidin is not generally well known, hence a review has been made of the literature dealing with the occurrence, the chemical nature, and the medical uses of this substance. The review discloses that this citrus glucoside, has been successfully used in the treatment of disorders caused by

[1] Nature and source of the vitamin material not stated but presumably hesperidin.

abnormal capillary fragility. Clinical and pharmacological evidence indicates this substance as an source of vitamin P

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Drug Extraction. XXIV. The Effect of the Length of Drug Column on the Efficiency of Percolation of Cinchona*

By William J. Husa† and Clifford T. Pacenta‡

Recent studies of drug extraction have shown that the method of forced percolation through a long column of drug has given more efficient extraction of several drugs than could be obtained by ordinary percolation (1, 2, 3). However, little information has been available regarding the effect of variations in the length of the drug column on the efficiency of extraction. Hence some research has been carried out on this point, using cinchona as representing an important drug which offers difficulties in extraction.

EXPERIMENTAL

Materials Used.—Yellow cinchona, U. S. P., in moderately coarse (No. 40) powder, assaying 7.31% alkaloids and 8.87% moisture was used. The menstrua employed were those prescribed in the U. S. P. X for fluidextract of cinchona.

Analytical Methods.—Alkaloidal content of the percolates was determined by the U. S .P. XI method for Compound Tincture of Cinchona, with a modification in the amount of sample taken. Asbestos fiber was used as the adsorbent. Moisture was determined by the U. S. P. XI method for drugs containing no constituents volatile at 100° C. To determine total extractive, 10 cc. of the liquid were evaporated to dryness on a water bath and then heated in an oven at 105° C. until the difference between two successive weighings did not exceed 10 mg.

Apparatus and Procedure.—The apparatus described by Husa and Huyck (1) was employed, the length of the drug column being increased or decreased by varying the number of tubes and elbows. The tubes were of Pyrex glass, each tube having a length of 91 cm. and an internal diameter of 2.5 cm. The drug was moistened with 25 cc. of Menstruum I per 100 Gm. of drug and packed, in sections, with moderately firm pressure, using 20 to 25 Gm. of drug for each packing portion. After being packed with drug, the tubes and elbows were joined together by means of rubber jackets and metal joint flanges. One end of the drug column was connected to a storage tank containing Menstruum II. This menstruum was forced through the drug column by means of air pressure obtained from the compressed air line, using a reducing valve and gage to secure the desired pressure. The percolate was collected in four equal fractions, the volume of each fraction amounting to 0.5 cc. for each Gm. of drug used. Each experiment was repeated and the data in the tables represent the averages of the duplicate experiments. No breaks in the drug column were observed in any of the experiments. The experimental conditions are given in Table I and the results are summarized in Table II.

DISCUSSION OF RESULTS

Table III shows the per cent of total alkaloids extracted in the first fraction, second fraction, and total of the first two fractions of percolate. Table IV shows the amount of total extractive obtained in 100 cc. of percolate in the first and second fractions, and also the total amount obtained in these two fractions.

The results in Tables III and IV indicate that the efficiency of extraction of alkaloids and total extractive is about the same for one and two tubes. The efficiency of extraction is greater when the drug column is lengthened by using three and five tubes, respectively.

Table I.—Experimental Data

	Experiments			
	A	B	C	D
Number of tubes used	1	2	3	5
Weight of drug in Gm.	300	600	950	1650
Approximate volume of packed drug in cc.	630	1295	1955	3325
Air pressure used	Up to 3 lb.	Up to 4 lb.	Up to 4 lb.	Up to 8 lb.
Average temperature	24.5° C.	24.5° C.	24.5° C.	23.5° C.
Time required for experiment (in hours)	29.5	98.1	210.5	449.2

* This paper is based on a thesis presented to the Graduate Council of the University of Florida by Clifford T. Pacenta, in partial fulfilment of the requirements for the degree of Master of Science in Pharmacy. Presented to the Scientific Section, A. PH. A., Detroit meeting, 1941.

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Table II.—Assay Results

Fraction	Gm. Total Extractive in Fraction	Gm. Alkaloids in Fraction	Per Cent Alkaloids Extracted
Experiment A			
1 (150 cc.)	39.22	6.45	29.43
2 (150 cc.)	28.87	4.84	22.08
3 (150 cc.)	19.80	3.41	15.56
4 (150 cc.)	11.31	2.20	10.04
Total (600 cc.)	99.20	16.90	77.11
Experiment B			
1 (300 cc.)	88.04	13.40	30.57
2 (300 cc.)	50.00	8.72	19.89
3 (300 cc.)	33.83	5.64	12.87
4 (300 cc.)	23.97	4.56	10.40
Total (1200 cc.)	195.84	32.32	73.73
Experiment C			
1 (475 cc.)	141.48	22.09	31.83
2 (475 cc.)	93.84	16.20	23.34
3 (475 cc.)	62.35	10.26	14.78
4 (475 cc.)	36.44	6.77	9.75
Total (1900 cc.)	334.11	55.32	79.70
Experiment D			
1 (825 cc.)	262.81	41.21	34.19
2 (825 cc.)	169.37	29.17	24.20
3 (825 cc.)	105.69	17.70	14.68
4 (825 cc.)	36.26	8.42	6.98
Total (3300 cc.)	574.13	96.50	80.05

Table III.—Efficiency of Extraction of Alkaloids

Experiment	Tubes and Elbows Used	Per Cent Alkaloids Extracted		
		Fraction 1	Fraction 2	Total Per Cent
A	1	29.43	22.08	51.51
B	2	30.57	19.89	50.46
C	3	31.83	23.34	55.17
D	5	34.19	24.20	58.39

Table IV.—Efficiency of Extraction of Total Extractive

Experiment	Tubes and Elbows Used	Gm. in 100 Cc. of Percolate		Total in First Two Fractions in Gm.
		Fraction 1	Fraction 2	
A	1	26.14	19.24	45.38
B	2	29.35	16.67	46.02
C	3	29.79	19.76	49.55
D	5	31.86	20.53	52.39

In general, the greater the length of drug column the higher is the per cent of total alkaloids extracted. It would seem that the length of the drug column is not the most important factor in the extraction of cinchona, since the longest drug column used yielded only about 58% of the total alkaloids in a percolate representing 1 cc. for each 1 Gm. of drug. In similar experiments with belladonna root (1), podophyllum (2) and ipomea (3), more than 99 per cent of the active constituents were obtained in the percolate representing 1 cc. per 1 Gm. of drug. The possibility of a combination between the alkaloids and tannins present in the drug, and subsequent precipitation in the tubes, may have some bearing on the results obtained.

The slightly lower efficiency of extraction of alkaloids with two tubes as compared with one tube (see Table III) may be considered as experimental error. However, some investigators have considered such variations as being real fluctuations, which were not readily explainable but which might be due to surface phenomena of adsorption, etc. (4, 5, 6).

The general results of the present study, indicating a somewhat greater efficiency of extraction with the longer drug columns, are in agreement with the results of J. U. Lloyd (7), who found that, in the extraction of cimicifuga, the longer the drug column the greater was the amount of extractive matter in the reserve percolate. Similarly Lefevre and Lee (8) found that increasing the length of the drug column increased the efficiency of extraction of krameria when the N. F. VI process of percolation was employed.

SUMMARY

A study was made of the effect of variations in the length of the drug column on the efficiency of extraction of cinchona in the process of forced percolation through a long column of drug. The general results indicate a somewhat greater efficiency of extraction with the longer drug columns.

The longest drug column used yielded only about 58% of the total alkaloids in a percolate representing 1 cc. for each Gm. of drug. In similar experiments with belladonna root, podophyllum and ipomea, more than 99% of the active constituents were obtained in a corresponding proportion of percolate.

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Book Reviews

Organic Reagents in Inorganic Analysis, by IBERT MELLAN, Ph.G., M.Sc., F.A.I.C. xxiii + 682 pages, 15 x 23 cm., index and appendix included. Structural formulas, graphs and tables. The Blakiston Company, Philadelphia, 1941. Price, $9.00.

Superior sensitivity and specificity of organic reagents over those of inorganic reagents used in various phases of qualitative and quantitative analysis have brought rapidly increasing interest on the part of the analyst. As a prelude to the main text of this book the author discusses, in twenty or more pages, fundamental theories and principles of organic reagents as they apply to the testing of inorganic radicles and compounds. Such topics as acidic hydrogen, coördination bond, chelated compounds, oxime and hydroxyl groups, etc., are considered in this short discussion.

Following the theoretical treatment is a group of approximately two hundred organic reagents, alphabetically listed, for testing inorganic chemicals. A short description of some physical and chemical properties is given under each reagent along with a bibliography, which, in some instances, contains as many as forty to fifty references.

In the final section of the book there are, alphabetically arranged, cations, anions and some inorganic compounds, all in alphabetical order, to which the organic reagents, previously presented, have application. Procedure for testing are given together with a bibliography at the end of each procedure.

In general the book is well organized, bibliographies are quite complete and up-to-date and the print is easy to read. However, the author has left much of the detail out of test procedures. The bibliographies are expected to take care of this deficiency. As for instance, in the test for small quantities of lead by the dithizone method the fact that special precaution as to purification of reagents, particularly dithizone, and cleansing apparatus before it is used in the test with a hot aqueous nitric acid solution has been entirely ignored. Although the author has stressed the sensitivity of some of these reagents in the first part of the text he has lost sight of it in a lack of detailed specifications where necessary. Some of these reagents can be used not only in spot tests and colorimetric qualitative analysis but have been adapted to very accurate quantitative work, again citing the dithizone as an example where quantities of lead have been accurately determined between four or five gamma.

This book serves only as a guide to the literature on the subject of organic reagents. Judging from the extent of the bibliographies this is what the author intended for it to be. In such a wide field he had no room for extra details in so small a space.—E. C. B.

The Glass Electrode. Methods, Applications, and Theory, by MALCOLM DOLE. First Edition, xv + 332 pages, illustrations. John Wiley and Sons, Inc., 440 Fourth Avenue, New York, New York, 1941. Price, $4.50.

This is the first book of its kind. With the introduction and perfection of thermionic tubes, the glass electrode has become a very useful tool in industrial work and in the determination of pH of those materials whose pH cannot be determined by hydrogen electrodes.

The book is both timely and authoritative. The author, from his own experience, has written a fine treatise with an extended bibliography of the construction, theory, advantages, limitations and application of the glass electrode which should be a valuable reference book for years. The chapters on the pH of living tissues and natural products are interesting and informative. Several chapters are devoted to the pH of unbuffered solutions, micro methods and automatic pH control which the author expertly employs to illustrate the wide application of the glass electrode.

Professor Dole devotes several chapters to the standardization of the pH scale by various methods, which though not a complete and ultimate answer, is nevertheless a valuable contribution to the efforts to arrive at a uniform practice in pH methods and applications. He suggests several materials, especially potassium acid phthalate which is supplied by the pH Standards Section of the National Bureau of Standards, for use in the calibration of glass electrodes. He also recommends for the same purpose borax, potassium tetraoxalate, and sodium and potassium phosphates, which are also being established as pH standards at the National Bureau of Standards, by potentiometric, colorimetric and conductometric methods. Professor Dole is to be congratulated on the completeness and unusual clarity of this book.—W. J. Hamor

pH and Electrotitrations, by I. M. KOLTHOFF and H. A. LAITINEN. Second Edition, ix + 190 pages, illustrations. John Wiley and Sons, Inc., 440 Fourth Avenue, New York, 1941. Price, $3.00.

This is the second edition of a well-known text intended for college seniors and graduate students in chemistry. It is noteworthy in that it brings together in one book four related subjects of importance in pure and applied chemistry. The first three parts deal with the applications of colorimetric, potentiometric and conductometric methods to a

variety of analytical procedures and pH determinations. The fourth part, not included in the first edition, gives clear introductions to voltammetry and amperometric titrations. These chapters, though brief, give the necessary background for understanding diffusion currents and the application of the polarograph to chemical analysis of inorganic and organic materials. All four parts are concisely presented with emphasis on experimental technique rather than theory. Sufficient theory is given, however, for a practical introduction to the various methods.

References to the literature are brief but adequate for beginners. Important revisions include new standard equations for the calculations of pH from hydrogen electrode measurements, a more detailed discussion of liquid junction potentials, newer values of ionization constants and a more extended use of thermodynamics in the theoretical derivations. The authors are to be commended on the clear and straightforward manner in which they present pertinent material.—W. J. Hamor

The Chemical Action of Ultraviolet Rays. By CARLETON ELLIS and ALFRED A. WELLS. Revised and enlarged edition by FRANCIS F. HEYROTH. 961 pages. 1941. New York: Reinhold Publishing Corp. $12.

The book contains 44 chapters divided into four parts as follows: Part I, The Sources of Ultraviolet Radiations; Part II, Photochemical Processes; Part III, Applications of Photochemistry to Industrial Products; Part IV, Applications of Ultraviolet Rays in Biology. The book represents an immense amount of labor in collecting literature. The author index contains over 6000 names and there are 38 printed pages of subject index. Certain explanatory chapters regarding the modern viewpoint have been added for readers who may not have had the advantage of recent advanced courses in physical chemistry. The volume represents a valuable work of reference for those interested in photochemistry.—A. G. D.

Report of Committee on Drug Addiction of the National Research Council, 1929–1941, and collected reprints, 1930–1941. xxx + 1580 pages, National Research Council, Washington, D. C. 1941.

Approximately thirty pages of this volume are devoted to the report of the Committee on Drug Addiction, in which the various activities of the Committee and its accomplishments are outlined. The remainder of the book is composed of reprints of papers in which there are reported chemical studies, pharmacological studies and clinical studies. There is a total of 153 reprints. This volume contains a mass of detailed information on practically all phases of drug addiction, and should serve as a valuable reference work for those seeking authentic information in this field.—A. G. D.

A Manual of the Literature of Chemistry, by GRACE RIGBY CAMERON. 77 pages. (c1940.) Baton Rouge, La.: Louisiana State University. 75 cents.

This guide to the literature of chemistry lists and discusses many of the more important reference works. The latter are classified in a somewhat arbitrary fashion and there is some repetition. The section on government publications is too incomplete and sketchy to be of much value. Critical discussion and completeness of information, so necessary for the beginner, are frequently lacking. The book also shows a lack of attention to detail in spelling It is believed, however, that it is worthy of a place in the chemist's library.—A. G. D.

Emulsions and Foams, by SOPHIA BERKMAN and GUSTAV EGLOFF. 591 pages. Reinhold Publishing Corp., 330 West 42nd Street, New York, N. Y., 1941. Price, $8.50.

This book is concerned primarily with the theory and applications of emulsions and, as such, it appears to be the most complete and comprehensive work of its kind published to date. The first chapter deals with the theory of emulsions and foams; the second, with emulsification; the third, with demulsification; the fourth, with practical knowledge of emulsions; and the fifth, with laboratory methods used in the examination of emulsions. The first hundred pages of the first chapter are devoted to a discussion of the views of many authors who have published on the theoretical aspects of emulsions and emulsification. An extensive bibliography is given. The volume contains much information on the practical applications of emulsions and should, therefore, be of value, particularly to technologists.—A. G. D.

Lange's Handbook of Chemistry, compiled and edited by NORBERT ADOLPH LANGE, Ph.D. Fourth Edition, 1935 pages. Handbook Publishers, Inc., Sandusky. Fab., $6.00.

This is the fourth edition of a reference volume for all who require ready access to the chemical and physical data used in laboratory work and in manufacturing. Much of the material in the previous editions has been completely rewritten and extended. The new tables and extension of old tables have added 85 pages to the book. The new tables are as follows: Synthetic Rubbers; Correction of the Boiling Point for Pressure; Calculation of the Boiling Point of Organic Compounds; Qualitative Spectrographic Analysis; Sensitive Lines of the Elements; Hammond's Cuprous Oxide and Copper Equivalents of Sugars; Conversion of Specific Gravity to Density; Azeotropic Mixtures; Tank and Pipe Capacities; Logarithms of Fractorial n. Factorials; Binominal Coefficients. The previous editions have proved to be of value to workers in in pharmaceutical laboratories and it is believed that the fourth edition with its changes in subject matter and new data will prove to be equally valuable.—A. G. D.

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